



Effects of oligotrophication and *Dreissena* littoral-pelagic coupling on littoral invertebrate and fish communities: insights from stable isotopes of archived samples

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Abstract Changing nutrient concentrations and introduction of non-native species affect the energy and nutrient fluxes in aquatic ecosystems. As a response, invertebrate communities could be altered, and ‘novel’ trophic communities could develop. Lake Constance (Europe) experienced pronounced nutrient variation caused by cultural eutrophication (prior 1980s) and oligotrophication (post-1980) and experienced many neobiota invasions. We used archived samples to investigate the impact of oligotrophication and invasive species on carbon and nitrogen isotope signatures of littoral macroinvertebrates during spring and autumn periods from 2000 to 2015 and of littoral fish species in 2018. Isotopic signatures of invertebrate and fish species were in the same range and showed similar seasonal differences and clear patterns in respect to littoral vs pelagic food sources and trophic level. Oligotrophication did not strongly alter isotopic values and only $\delta^{15}\text{N}$ values of invertebrates declined weakly with oligotrophication. In contrast, interannual variability of stable isotope signatures

was related to abundances of the invasive *Dreissena polymorpha* suggesting interannual differences in the importance of benthic-pelagic coupling due to *Dreissena* pseudofaeces for the nutrition of littoral invertebrates. This study illustrates how stored samples can be used to detect the importance of oligotrophication and benthic-pelagic coupling on the stable isotope signatures of a benthic community.

Keywords *Dreissena polymorpha* · Biodeposition · Community niche width · Littoral resources · Lake Constance · Benthic-pelagic coupling

Introduction

Energy fluxes within food webs as well as between ecosystem habitats can be strongly altered by environmental changes such as eutrophication of lakes (Vadeboncoeur et al., 2003; Chandra et al., 2005) as well as by the invasion of neozoans (Karatayev et al., 1997). For example, eutrophication has been shown to shift lake primary production from benthic producers towards pelagic producers (Vadeboncoeur et al., 2003). The invasion of filtering mussels, such as *Dreissena polymorpha* (Pallas, 1771), has been shown to have opposite effects as their feeding reduces biomass and productivity of primary producers (Higgins & Vander Zanden, 2010; McEachran et al., 2019) and via deposition of pseudofaeces provides an additional

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food source for benthic consumers (Gergs & Rothhaupt, 2008; Ozersky et al., 2012).

Stable isotopes have been shown to be a powerful tool to investigate food web dynamics in a variety of study systems, such as estuaries, the Antarctic Ocean, seabird rookeries and humans (Wada et al., 1991). They are especially important tools to study energy fluxes in food webs as well as between ecosystem compartments or habitats (Yoshioka et al., 1994; Grey et al., 2001; Jones & Waldron, 2003).

The consequences of nutrient changes for isotopic signatures have been documented using space-for time substitutions (Premke et al., 2010), paleolimnological investigations (Guiry, 2019), or studies using archived material such as fish scales (Perga & Gerdeaux, 2005). There are, however, no studies examining the long-term effects of nutrient changes for isotopic signatures in littoral food webs. The influence of *Dreissena* on isotopic signatures on littoral food webs is also well studied using space-for time substitutions (McEachran et al., 2019), or comparing lakes before and after *Dreissena* invasion (Ozersky et al., 2012). These two studies documented increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in secondary invertebrate consumers and fish with higher *D. polymorpha* abundance and concluded that the mussel enforced a higher reliance on littoral energy sources (Ozersky et al., 2012; McEachran et al., 2019). Nevertheless, effects of temporal variations in *Dreissena* abundance on littoral isotopic signatures have rarely been studied. A study over two consecutive years in Lake Constance showed that mass mortality of *Dreissena* during a severe winter led to higher carbon and lower nitrogen isotopic signatures of gammarids possibly due to a reduction of *Dreissena* pseudofaeces and subsequent alternative feeding on littoral epilithon (Gergs et al., 2011).

Seasonal changes of environmental conditions and of primary productivity also result in seasonal changes in isotopic signatures of primary producers with more negative $\delta^{13}\text{C}$ values in winter (^{13}C -depleted) and less negative $\delta^{13}\text{C}$ values in summer (^{13}C -enriched) (Zohary et al., 1994; Woodland et al., 2012; Visconti et al., 2014). In contrast, $\delta^{15}\text{N}$ values display the inverse pattern of summer depletion and winter enrichment (Bunn & Boon, 1993; Woodland et al., 2012).

Here, we investigate changes in stable isotope signatures of littoral macroinvertebrates during 15 years of

oligotrophication and interannual variability of *Dreissena* abundances in deep perialpine Lake Constance using the stable isotope ratios of carbon and nitrogen. We furthermore compare the range of stable isotope signatures of invertebrates with the signature of various fish species caught in the littoral zone. $\delta^{13}\text{C}$ may be used to determine the potential energy sources of consumers (Vander Zanden & Rasmussen, 2001) as littoral producers are more enriched (more positive) in $\delta^{13}\text{C}$ compared to pelagic producers (more negative) $\delta^{13}\text{C}$ values (France, 1995). Nitrogen-isotope ratios ($\delta^{15}\text{N}$) are used to evaluate trophic relationships in communities and trophic positions of organisms due to step-wise enrichment (3.0–4.0‰) of $\delta^{15}\text{N}$ from prey to predator (Vander Zanden & Rasmussen, 2001). However, littoral producers have also been observed to be more enriched in $\delta^{15}\text{N}$ compared to pelagic producers (Zanden & Rasmussen, 1999). In addition, isotopic signatures of both elements might be influenced by oligotrophication as reduced primary productivity might release producers from carbon and/or nitrogen limitation thereby shifting their isotopic signatures to more negative values (Peterson & Fry, 1987; Schindler et al., 1997; Gu, 2009).

Using isotopic signatures of eight macroinvertebrate species sampled during the period 2000–2015 and ten fish species sampled in 2018, we test the following hypotheses:

- (1) Isotopic signatures of macroinvertebrate and fish will differ significantly between species and season.
- (2) Oligotrophication will result in declining productivity in the littoral zone and consequently in a decrease of both carbon and nitrogen isotopic signatures of littoral macroinvertebrates.
- (3) High *Dreissena* abundances will result in high biodeposition rates and thus in more negative carbon isotopic signatures and higher nitrogen isotopic signatures of littoral macroinvertebrates.
- (4) Isotopic signatures of macroinvertebrate species will be differently affected by oligotrophication and *Dreissena* abundances.

Material and methods

Sample collection

Sampling of littoral macroinvertebrates took place at ‘Litoralgarten’, a sampling site close to the Limnological Institute, University of Konstanz located at the south-western shore of the Überlingen Basin of Upper Lake Constance (47° 41.5′ N, 9° 12.20′ E). The shore of the sampling site is characterised by wide boulders and silty sand with loosely packed stony overlay (Baumgärtner et al., 2008) which is a typical and dominant littoral substrate in Lake Constance (e.g. Fischer & Eckmann, 1997; Baumgärtner et al., 2008; Gergs et al., 2009). We consider our samples representative for this habitat type in the lake. Sampling took place in spring (March or April) and autumn (September, October, November).

A suction sampler was used to collect benthic invertebrates in three different depths (MLW, MLW-1 m and 40 cm below the water surface) of the littoral zone. In the laboratory, sampled stones were rinsed and brushed to remove all individuals, while fine sediment was sieved to pick out individuals. Each sample was preserved in 70% ethanol (see Baumgärtner et al., 2008; Gergs & Rothhaupt, 2015 for more details). These samples were used to calculate densities of *Dreissena polymorpha* and to select invertebrates for stable isotope analyses.

Eight benthic invertebrate taxa were selected because they are among the most dominant taxa in the littoral invertebrate community of Lake Constance and perform different feeding modes: the native taxa *Bithynia tentaculata* (Linnaeus, 1758) (filter feeding and grazing), Chironomidae (different feeding modes), *Ecnomus tenellus* (Rambur, 1842) (predation), and *Tinodes waeneri* (Linnaeus, 1758) (grazing) and the invasive species *Dikerogammarus villosus* (Sowinsky, 1894) (predation), *Dreissena polymorpha* (Pallas, 1771) (filter feeding), *Gammarus roeselii* Gervais, 1835 (grazing), and *Limnomyxis benedeni* Czerniavsky, 1882 (different feeding modes).

Additionally, littoral fish were caught in spring (i.e. May, June) and summer (i.e. August, September) 2018. Most fish (75.4%) were caught at the same sampling site as was used for the invertebrate samples. As we were not successful in catching whitefish and only one individual of ruffe at the ‘Litoralgarten’,

these fish were caught at two other sites with, however, similar habitat characteristics (Meersburg, and Langenargen). Sampling was carried out with beach seines (length 16 m, height 2 m, mesh size 4 mm [bar mesh]) hauled from 1 m water depth to the shoreline and carried out under a permit from the Regierungspräsidium Tübingen (Aktenzeichen 33-4/9220.51-3). In total, 138 individuals from 10 different fish species were caught, 6 species in both seasons [chub: *Squalius cephalus* (Linnaeus, 1758), dace: *Leuciscus leuciscus* (Linnaeus, 1758), Eurasian perch: *Perca fluviatilis* Linnaeus, 1758 (hereafter referred to as perch), loach: *Barbatula barbatula* (Linnaeus, 1758), roach: *Rutilus rutilus* (Linnaeus, 1758), and ruffe: *Gymnocephalus cernua* (Linnaeus, 1758)], bleak: *Alburnus alburnus* (Linnaeus, 1758), three-spined stickleback (*Gasterosteus aculeatus* Linnaeus, 1758) (hereafter referred to as stickleback), and whitefish (*Coregonus* spp.) only in spring, Bream [*Abramis brama* (Linnaeus, 1758)] only in summer. Fish were immediately euthanized and stored on ice; in the lab, muscle tissue was taken from the caudal fin of each fish and frozen until further analysis.

Total phosphorus concentrations measured during the winter mixing period (TP_{mix}) were provided by the Institute for Lake Research, Langenargen and calculated as volumetrically weighted TP values during March of each year (which is usually the month of full mixing in Lake Constance). *Dreissena polymorpha* abundance data were calculated from samples collected at ‘Litoralgarten’ and published in Gergs & Rothhaupt (2015). Sampling methods of *D. polymorpha* abundance data are detailed in Gergs & Rothhaupt (2015).

Sample analysis: stable isotopes

For performing stable isotope analysis, we took individuals from ten sampling years: 2000, 2002, 2003, 2005, 2007, 2008, 2010, 2012, 2013 and 2015 allowing for a gap of a most one year between study years. We pooled several individuals for one sample. Whenever possible we prepared four replicates per species, season and year. Storage in ethanol could affect the stable isotope measurements of our samples as ethanol may have leached out lipids from the stored organisms (Kelly et al., 2006; Syväranta et al., 2008). Levels of ¹³C in lipids are depleted relative to whole organism or tissue ¹³C values, and therefore,

variations in lipid content may skew isotope values (Post et al., 2007; Rothhaupt et al., 2014). For this reason, lipid extraction is usually recommended prior to isotope analysis (Peterson & Fry, 1987; Rothhaupt et al., 2014; Kling et al., 2016). To standardise the lipid content of our samples, we performed lipid extraction by soaking in a 2:1 chloroform–methanol solution for 24 h, then rinsing in distilled water, before further preparation for conducting stable isotope analysis. Dried and pulverised samples were weighed (ca. 0.7 mg) in tin cups to the nearest 0.001 mg, using a micro analytical balance. Samples were combusted in a vario PYRO cube elemental analyser (Elementar Analysensysteme, Germany) at 1120 °C. Resulting CO₂ and N₂ were separated by gas chromatography and passed into an IsoPrime isotope ratio mass spectrometer (IRMS; Isoprime Ltd., Manchester, UK) for determining ¹³C/¹²C or ¹⁵N/¹⁴N ratios. All stable isotope values (δ¹³C or δ¹⁵N) are reported in the δ notation (per mill), where δ = (1,000 × [R_{sample}/R_{standard}] - 1) ‰; relative to the Pee Dee Belemnite (PDB) for carbon and atmospheric N₂ for nitrogen in parts per thousand deviations (‰). Prior to statistical analyses, carbon isotope measurements were adjusted to account for the Suess effect, i.e. the effect of increasing fossil-derived CO₂ on atmospheric δ¹³C values (Verburg, 2007). Adjustment was done based on the 3rd order polynomial equation given in Verburg (2007), which corresponds to adding 0.0369 to the δ¹³C values for each year prior to 2015. All data of this study are found in Sabel et al. (2024).

Statistical analysis

All calculations were conducted using R (R Core Team, 2020). For invertebrate samples, we used linear regression models to investigate dynamics of δ¹³C and δ¹⁵N mean values (i.e. dependent variables) with various combinations and twofold interactions of the independent variables species, season (i.e. spring and autumn), sampling depth, TP_{mix} and log-transformed *D. polymorpha* mean abundance (m²) per season and year. We used Akaike's Information Criterion (AIC) to rank the different models, and show the five best models (i.e. the models with the lowest AIC) for each element. We additionally ran Type II ANOVA for the best models to identify significant independent variables and interactions using the R package car (Fox &

Weisberg, 2011). Partial residuals for predictor variables and interactions in multivariate models were visualised using the 'effects' package (Fox, 2003). For fish samples, we used linear regression models and type II ANOVA to investigate differences of δ¹³C and δ¹⁵N values between different seasons (i.e. spring and summer) and fish species.

Results

During the study period, TP_{mix} concentrations decreased from 14.3 µg l⁻¹ in 2000 to values between 5.8 µg l⁻¹ and 7.9 µg l⁻¹ after 2006 (Fig. 1A). Mean abundance of *Dreissena polymorpha* varied between ~42,000 individuals/m² (autumn samples in 2007) and individuals/m² (spring samples in 2006), but did not show a significant trend during the study period (*P* = 0.722). Throughout the sampling period, *Dreissena* abundance in autumn was higher than in spring (Fig. 1B).

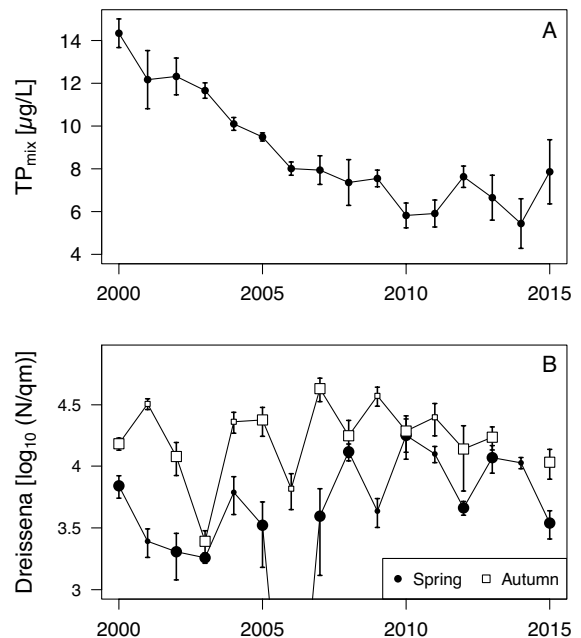


Fig. 1 A Development of total phosphorous (TP_{mix}) concentrations (µg l⁻¹) during winter mixing of Lake Constance (mean ± SE) and B of mean (± SE) abundances of *Dreissena polymorpha* (N m⁻²) during the study period. *Dreissena* abundances are shown for spring (filled circle) and autumn (empty rectangle) samples. Enlarged symbols indicate the years in which stable isotope values (δ¹³C and δ¹⁵N) were measured

Carbon isotopes ($\delta^{13}\text{C}$)

For invertebrate $\delta^{13}\text{C}$, no single best model emerged and AIC of the best three models differed less than two. These three models suggested significant differences for species and season as well as for the interaction between the two variables (Table 1, Fig. 2A). *Dreissena polymorpha* ($-30.34 \pm 0.25\text{‰}$ in spring, $-29.74 \pm 0.17\text{‰}$ in autumn) and *Limnomysis benedicti* ($-29.59 \pm 0.15\text{‰}$ in spring, $-29.02 \pm 0.41\text{‰}$ in autumn) had significantly lower mean $\delta^{13}\text{C}$ values than the other investigated taxa. Mean $\delta^{13}\text{C}$ values of Chironomidae, *Dikerogammarus villosus*, *Gammarus roeselii* and *Tinodes waeneri*, but not of other taxa, were significantly higher in autumn compared to spring (Fig. 2A).

TP_{mix} and *Dreissena polymorpha* abundance were not consistently included in these best models: TP_{mix} and the interaction between TP_{mix} and season were included in one of the three best models; *D. polymorpha* abundance was included in two of three best models, and the interaction between *D. polymorpha* abundance and season was included in one of the three best models. The interactions between species identity and TP_{mix} , or *D. polymorpha* abundance were not included in the three best models for $\delta^{13}\text{C}$, i.e. we found no evidence for species-specific effects of oligotrophication or *D. polymorpha* biodeposition on invertebrate $\delta^{13}\text{C}$. Likewise, sampling depth did not contribute to the best models. According to the best model, there was no significant relationship between TP_{mix} and carbon signatures (Fig. 3A, B), albeit the inclusion of the interaction between TP_{mix}

and season in the best model (Table 1) might suggest a weak response to oligotrophication in the spring samples (Fig. 3B). In contrast, increasing *D. polymorpha* abundances were significantly associated with decreasing carbon signatures (Fig. 3C).

The range of mean $\delta^{13}\text{C}$ values of the littoral fish species (Fig. 2B) was almost as large as the mean range of the macroinvertebrates $\delta^{13}\text{C}$ (Fig. 2A). Highest $\delta^{13}\text{C}$ were observed for the cyprinid species bream, loach, chub and roach in summer ($\sim -23\text{‰}$) and lowest $\delta^{13}\text{C}$ for whitefish, stickleback, perch and bleak in spring ($\sim -29\text{‰}$, Fig. 2B). Across all species, spring $\delta^{13}\text{C}$ values were lower than summer $\delta^{13}\text{C}$ values (mean difference for those species caught in both seasons: 2.4‰ , $F=88.233$, $df=1$, 104 , $P<0.001$) and fish species differed significantly regarding their $\delta^{13}\text{C}$ values ($F=19.752$, $df=9$, 120 , $P<0.001$). However, no species-specific seasonal differences could be detected within the six species which were caught in both seasons (interaction species:season, $F=1.662$, $df=6$, 120 , $P=0.136$).

Nitrogen isotopes ($\delta^{15}\text{N}$)

In contrast to $\delta^{13}\text{C}$, there was one clearly superior model explaining $\delta^{15}\text{N}$ variability, with AIC more than nine units lower compared to the 2nd best model (Table 2). This model consists of the main effects of four independent variables (species, season, TP_{mix} and *Dreissena polymorpha* abundance), but did not include any interactions. Hence, we found no support for species-specific seasonal variability of macroinvertebrate $\delta^{15}\text{N}$.

Table 1 Linear regression models with various combinations and interactions between independent variables (species, season, TP_{mix} and log-transformed mean *Dreissena polymorpha*

abundance (*D.p.Abu*) per sampling month and year) were calculated for the mean $\delta^{13}\text{C}$ values of investigated taxa

Carbon models	df	dAIC	Weight
C1) Season + Species + TP_{mix} + <i>D.p.Abu</i> + Season:Species + Season:TP_{mix}	20	0	0.38
C2) Season + Species + Season:Species	17	1.0	0.23
C3) Season + Species + <i>D.p.Abu</i> + Species:Season + Season:<i>D.p.Abu</i>	19	1.3	0.2
C4) Season + Species + TP_{mix} + <i>D.p.Abu</i> + Species:Season + Season:TP_{mix} + Season:<i>D.p.Abu</i>	21	2.0	0.141
C5) Season + Species + TP_{mix} + Season:Species + Season:TP_{mix}	19	4.6	0.038

The best five models are listed, starting with the model with the highest weight and, therefore, the best fit for the data according to the Akaike Information Criterion (AIC). dAIC indicates the differences between the AIC of the different models and the best model. Significant variables and interactions ($P<0.05$) are shown in bold; variables and interactions showing a tendency ($P<0.1$) are underlined

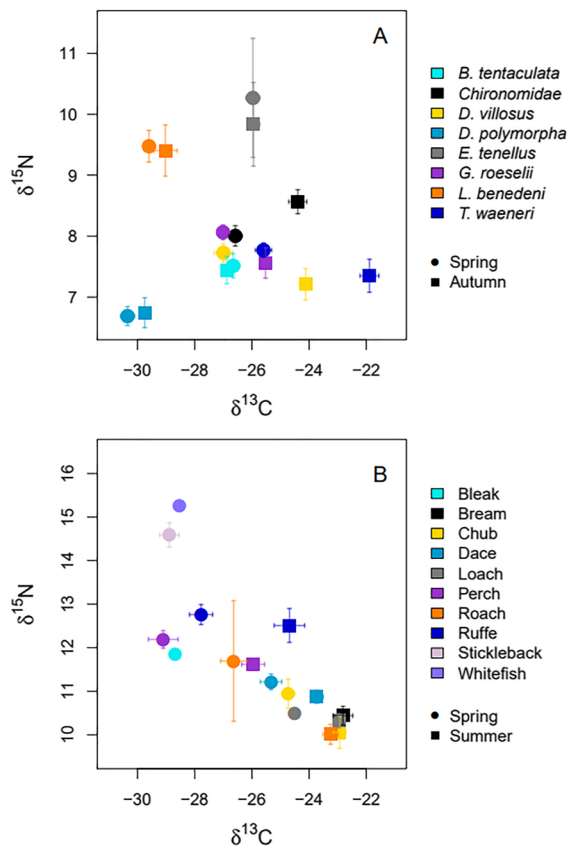


Fig. 2 **A** Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (\pm SE) of eight investigated invertebrate taxa (*B. tentaculata*, Chironomidae, *D. villosus*, *D. polymorpha*, *E. tenellus*, *G. roeselii*, *L. benedeni* and *T. waeneri*). Colours indicate species and shapes represent sampling seasons (i.e. circle=spring, rectangle=autumn). **B** Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (\pm SE) of ten littoral fish taxa (bleak (*A. alburnus*), bream (*A. brama*), chub (*S. cephalus*), dace (*L. leuciscus*), loach (*B. barbatulus*), perch (*P. fluviatilis*), roach (*R. rutilus*), ruffe (*G. cernua*), stickleback (*G. aculeatus*), whitefish (*Coregonus* spp.)). Colours indicate species and shapes represent sampling seasons (i.e. circle=spring, rectangle=summer)

Ecnomus tenellus ($10.27 \pm 0.98\text{‰}$ in spring, $9.84 \pm 0.69\text{‰}$ in autumn) and *Limnomysis benedeni* ($9.48 \pm 0.26\text{‰}$ in spring, $9.4 \pm 0.42\text{‰}$ in autumn) had significantly higher $\delta^{15}\text{N}$ mean values than the other investigated taxa (Fig. 2A). Across all species, average $\delta^{15}\text{N}$ values were lower in autumn compared to spring.

Mean $\delta^{15}\text{N}$ values significantly increased with increasing TP_{mix} values (Fig. 4A) and also with higher *D. polymorpha* abundance (Fig. 4B). The interactions between species identity and TP_{mix} , or *D. polymorpha*

abundance were not included in the best models for $\delta^{15}\text{N}$, i.e. we found no evidence for species-specific effects of oligotrophication or *D. polymorpha* bio-deposition on invertebrate $\delta^{15}\text{N}$. Likewise, sampling depth did not contribute to the best model.

Mean $\delta^{15}\text{N}$ values of the littoral fish species ranged between 10.5‰ (dace) and 15.3‰ (whitefish) in spring and between 10.0‰ (roach) and 12.5‰ (ruffe) in summer (Fig. 2B). In spring, whitefish and stickleback $\delta^{15}\text{N}$ ($\sim 15\text{‰}$) were clearly elevated (2–3‰ higher) compared to those of other species. Across all species, spring $\delta^{15}\text{N}$ values were higher than summer $\delta^{15}\text{N}$ values (mean difference for species caught in both seasons: 0.7‰ , $F=12.978$, $\text{df}=1, 104$, $P<0.001$). $\delta^{15}\text{N}$ values significantly differed between species ($F=25.787$, $\text{df}=9, 120$, $P<0.001$), but we could not detect significant species-specific seasonal differences (species:season interaction: $F=1.193$, $\text{df}=6, 120$, $P=0.315$).

Discussion

Changes in trophic status as well as invasion of neobiota might strongly alter energy fluxes and the trophic structure of ecosystems. Stable isotopes are an important tool to identify such changes, as they respond sensitively to changes in ecosystem productivity (Vadeboncoeur et al., 2003; Chandra et al., 2005), food web relationships (Hecky & Hesslein, 1995; Crawford et al., 2008; Phillips et al., 2014), and habitat coupling within ecosystems (Hershey et al., 2006; Gergs et al., 2009). However, the sensitivity of isotopic signatures to such a multitude of processes complicates their interpretation in respect to long-term changes especially when multiple processes change simultaneously.

Here, we analysed stable isotope signatures of components of the littoral food web of Lake Constance throughout a period of 15 years based on samples stored in 70% ethanol. Ethanol preservation is known to extract lipids and therefore slightly increase $\delta^{13}\text{C}$ values ($0.74 \pm 0.14\text{‰}$), while $\delta^{15}\text{N}$ values of macroinvertebrates are less impacted ($0.28 \pm 0.12\text{‰}$) (Syväranta et al., 2008). Syväranta et al. (2008) found that there is no relationship between preservation time and the effect of preservation on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of macroinvertebrates up to 12 months of preservation. However, our samples have been preserved

Fig. 3 Partial residuals and model fits (\pm SE) for the effects of **A** TP_{mix} in spring, **B** TP_{mix} in autumn and **C** log-transformed *Dreissena* abundance within the best model for mean $\delta^{13}C$ values identified by AIC (Table 1). Note that the TP_{mix} :season interaction contributed to the best model but was not significant ($F_{1,122} = 1.8$, $P < 0.19$)

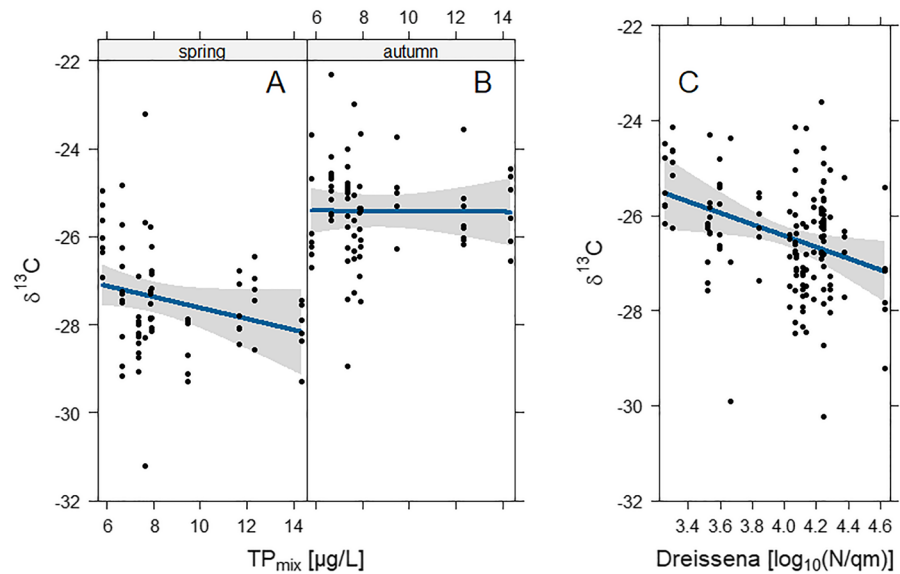


Table 2 Linear regression models with various combinations and interactions between independent variables (species, season, TP and log-transformed mean *Dreissena polymorpha*

abundance (*D.p.Abu*) per sampling month and year) were calculated for the mean $\delta^{15}N$ values of investigated taxa

Nitrogen models	df	dAIC	Weight
N1) Season + Species + TP_{mix} + <i>D.p.Abu</i>	12	0	0.967
N2) Season + Species + TP_{mix} + <i>D.p.Abu</i> + Species:TP_{mix} + Season:TP_{mix} + <i>D.p.Abu</i>:TP_{mix}	21	9.2	0.01
N3) Season + Species + TP_{mix} + <i>D.p.Abu</i> + Species:Season + TP_{mix}:Season + <i>D.p.Abu</i>:Season	21	10.1	0.006
N4) Season + Species + TP_{mix} + <i>D.p.Abu</i> + TP_{mix}:Species + Season:Species + <i>D.p.Abu</i>:Species	33	10.3	0.006
N5) Season + Species + TP_{mix} + Species:Season + TP_{mix}:Season	19	11.0	0.004

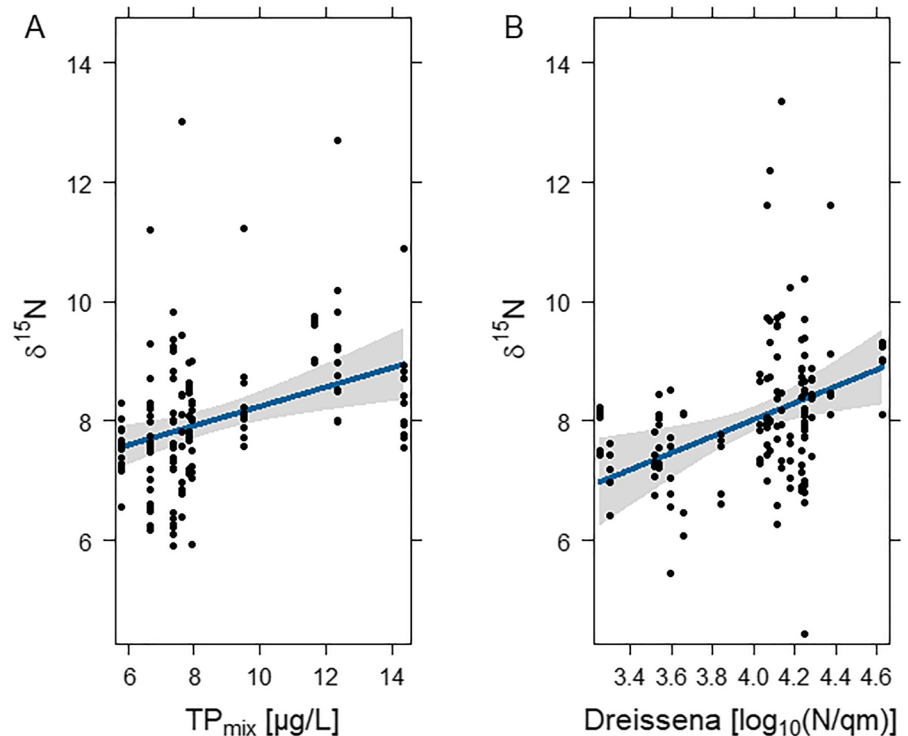
The best five models are listed, starting with the model with the highest weight and therefore the best fit for the data according to the Akaike Information Criterion (AIC). dAIC indicates the differences between the AIC of the different models and the best model. Significant variables and interactions ($P < 0.05$) are shown in bold; variables and interactions showing a tendency ($P < 0.1$) are underlined

for up to 18 years and we cannot exclude possible effects especially on $\delta^{13}C$ values over a longer time period (> 12 months). Therefore, we additionally extracted lipids (see material and methods), which is recommended by several authors (Peterson & Fry, 1987; Rothhaupt et al., 2014; Kling et al., 2016), to reduce possible effects of ethanol storage duration on $\delta^{13}C$.

In order to investigate the role of the two non-native species that were introduced in the lake during our study period (*Dikerogammarus villosus* and *Limnomysis benedeni*) for long-term dynamics of isotopic signatures, we also ran models without the isotopic signatures of these two species (stats not shown). For $\delta^{13}C$, the best model did not include

the interaction between season and TP_{mix} suggesting that the $\delta^{13}C$ values of the two invasive and plankton feeding species contributed to the small differences in response of $\delta^{13}C$ to TP_{mix} between seasons. The best model for $\delta^{15}N$ also changed slightly as the independent variable ‘season’ was not significant anymore indicating that these two species contributed to the observed mean difference between spring and autumn $\delta^{15}N$ signatures. Hence, both isotopes signatures indicate larger seasonal differences between pelagic and littoral feeding invertebrates. However, there is no indication that the isotope signals of these two species were the main drivers of the observed long-term changes in isotopic values.

Fig. 4 Partial residuals and model fits (\pm SE) for the effects of **A** TP_{mix} and **B** log-transformed *Dreissena* abundance within the best model for mean $\delta^{15}N$ values identified by AIC (Table 2)



As our data are based on preserved samples of macroinvertebrates, we do not have long-term data of potential food sources of macroinvertebrates. However, previous studies showed that $\delta^{13}C$ in POM of Lake Constance is significantly lower than $\delta^{13}C$ of epilithon, whereas there is less difference between those food sources regarding $\delta^{15}N$ (Gergs et al. 2011).

Hypothesis 1: stable isotopes as indicators of food sources

The significantly lower carbon signatures of *Dreissena polymorpha* and *Limnomysis benedeni* compared to other invertebrates support their proposed nutrition from pelagic sources, i.e. respectively, phytoplankton and zooplankton. In contrast, other macroinvertebrates fed on littoral food sources, or a mixture of littoral and pelagic food sources; the latter possibly derived from *Dreissena* pseudofaeces (Gergs et al., 2009). In contrast to carbon signatures, the differences between seston and epilithic $\delta^{15}N$ were rather small in a previous study (Gergs et al. 2011), suggesting that the observed differences in $\delta^{15}N$ between macroinvertebrates are not strongly influenced by the origin of the food

source, i.e. pelagic versus epilithic, but result from differences in trophic level. As for macroinvertebrates, carbon signatures of fish suggest pelagic food sources for some species (whitefish, stickleback, perch and bleak), and littoral food sources, or a mixture of both, for other species. Highest $\delta^{15}N$ signatures were observed for two species (whitefish and stickleback), which carbon signatures indicate pelagic food sources. As both fish species are planktivores in Lake Constance (Ogorelec et al., 2022), the high $\delta^{15}N$ in these spring samples may result from catabolic processes during the previous winter as suggested for whitefish in another perialpine lake (Perga & Gerdeaux, 2005). Catabolic processes might be especially important for stable isotope values of planktivores during winter, as in deep lakes such as Lake Constance the most important pelagic food, i.e., large zooplankton species, strongly declines during winter (Straile, 2015). In contrast, food sources in the littoral may be—due to the longer life cycles of e.g. benthic insects—more abundant also in winter (Wetzel, 2001). In case of sticklebacks, Gugele et al. (2023) suggested that $\delta^{15}N$ may be further increased due to feeding on larvae and juveniles of whitefish.

Seasonal changes in stable isotope signatures observed for macroinvertebrates and fish also mimic known patterns of pelagic food sources in Lake Constance. $\delta^{13}\text{C}$ values of sedimenting POM increased from early May values of -33.0‰ towards -30.0‰ by the end of September (Fuentes et al., 2013). Likewise, $\delta^{13}\text{C}$ (from -35 to -30.5‰) and $\delta^{15}\text{N}$ (from 5.5 to 7.5‰) of *D. polymorpha* growing in upper water layers on a cable in the pelagic zone increased from May towards August (Yohannes et al., 2014).

Hypothesis 2: stable isotopes as indicators of changing productivity

TP_{mix} was included as a predictor in models explaining variability for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ suggesting that littoral productivity declined during the study period. However, TP_{mix} only contributed significantly to the models for $\delta^{15}\text{N}$ and not for $\delta^{13}\text{C}$. Also, the tendency for an interaction between TP_{mix} and season, which resulted from an approximately 1‰ increase with decreasing TP_{mix} in spring samples of $\delta^{13}\text{C}$, vanished when excluding the two species feeding from pelagic sources. The decrease of $\delta^{15}\text{N}$, which could be expected due to the results of Gu (2009), was also rather small ($\sim 1\text{‰}$) suggesting that overall the productivity decline in the littoral zone was not very strong during the study period. Furthermore, we cannot fully exclude a potential confounding effect of climate warming on stable isotope dynamics, although we think that any effect of warming is likely small. While $\delta^{13}\text{C}$ of consumers have been shown to increase and $\delta^{15}\text{N}$ to decrease with warmer temperatures (Power et al., 2003), the differences in experimental temperature in these studies strongly exceed the temperature increase observed during the study period. For example, Power et al. (2003) showed that a ~ 14 °C increase (from 12.8 °C to 26.5 °C) of water temperature resulted in an approx. 1.4‰ increase in $\delta^{13}\text{C}$, and a decrease of approx. 2.2‰ $\delta^{15}\text{N}$ of *Daphnia magna*. However, surface temperatures in Lake Constance increased considerably less during the study period (< 0.5 °C, IGKB, 2020), which suggests that warming is probably not a major determinant of the changes in stable isotope composition observed in this study.

$\delta^{13}\text{C}$ of scales of pelagic planktivorous whitefish declined approximately by 3‰ during 1983 and 2001 in Lake Constance (Gerdeaux & Perga, 2006). This

indicates that in the pelagic zone a major productivity decline did occur already before our study period (see also Milan et al., 2022). Stable catch per unit effort of the littoral fish community in Lake Constance during roughly our study period (i.e. from 1997 to 2013; Sabel et al., 2020) also suggests no strong declines in littoral productivity. Furthermore, phosphorous remobilisation from littoral sediments (Güde et al., 2000), and/or improved light conditions for benthic producers due to the decline of phytoplankton (Vadeboncoeur et al., 2002) might have also contributed to a rather small productivity response in the littoral zone during our study period.

Hypothesis 3: stable isotopes as indicators of habitat coupling

Two of the four invasive species analysed in this study remarkably increased the overall range of both carbon and nitrogen isotopes and thus the niche range of the littoral invertebrate community. The invasion of *Dreissena polymorpha* expanded the $\delta^{13}\text{C}$ values strongly towards more negative values, whereas the invasion of *Limnomyia benedeni* expanded the range of $\delta^{15}\text{N}$ values at low $\delta^{13}\text{C}$ values. While both invasive species feed on pelagic food resources resulting in low $\delta^{13}\text{C}$ values, feeding on zooplankton by *L. benedeni* explains the higher $\delta^{15}\text{N}$ values compared to most other investigated species (Sprung, 1989; Fink et al., 2012; Fink & Harrod, 2013). Thus, these two species provide a link between the littoral and the pelagic zone at two trophic levels. Moreover, other studies already showed that sestonic, biodeposited material of *D. polymorpha* contributes to the biomass and production of the littoral benthos that also induces an isotopic shift towards lower $\delta^{13}\text{C}$ values (Ricciardi et al., 1997; Gergs et al., 2009; Ozersky et al., 2012). As predicted in our third hypothesis, $\delta^{13}\text{C}$ values decreased significantly with increasing abundances of *D. polymorpha* suggesting that pelagic derived biodeposition served as an additional food source for the investigated taxa. Biodeposition material of *D. polymorpha* has been experimentally demonstrated to provide an additional food source for other invertebrates such as gammarids (i.e. *Dikergammarus villosus* and *Gammarus roeselii*) as well as chironomids (Gergs et al., 2011). Excluding isotopic values of *D. polymorpha* from statistical analysis did not lead to altered results in model choice or changes

in relationships of independent variables, suggesting that dynamics of overall macrobenthos $\delta^{13}\text{C}$ values were not driven by isotopic values of the mussel. Hence, our data support our prediction that changes in *Dreissena* abundances alter the importance of pelagic food sources for the littoral food web.

Similar to $\delta^{13}\text{C}$ values, changes in $\delta^{15}\text{N}$ values support our hypotheses and show a significant increase with higher *Dreissena polymorpha* abundance. This also supports previous findings that $\delta^{15}\text{N}$ values of amphipods increased with increasing *Dreissena* abundance (Gergs et al., 2011). However, in our study, the increase in $\delta^{15}\text{N}$ with high *Dreissena* abundances was less pronounced ($\sim 1.00\text{‰}$) compared to the shift ($\sim 8.00\text{‰}$) observed previously in Lake Constance when comparing two study years (Gergs et al., 2011). This study suggested that *D. polymorpha* may have used its own pseudofaeces—after partial bacterial decomposition and re-suspension of the particles in the water column (Garton et al., 2005)—as an alternative food source that led to further increased *D. polymorpha* and pseudofaeces $\delta^{15}\text{N}$. We could not find a similar strong increase $\delta^{15}\text{N}$ and thus found no support for the proposed food web response.

Hypothesis 4: species-specific differences in the effects of oligotrophication and *Dreissena* abundance

Due to differences in food sources and feeding modes (i.e. filter feeding, grazing and predation) between the investigated species, species-specific differences of the effects of oligotrophication and *D. polymorpha* abundance on carbon and nitrogen signatures were expected. However, statistical models did not provide support for species-specific responses to environmental drivers, which might be due to the overall small effect sizes we did observe.

Conclusion

We used stable isotopes of macroinvertebrates from samples stored in ethanol to study the effects of oligotrophication and *Dreissena* habitat coupling on littoral invertebrates in a large and deep perialpine lake. We showed that variability in *Dreissena* abundances and possibly TP_{mix} affected macroinvertebrate stable isotope signatures during our study period. However, shifts in isotope signatures with oligotrophication

were rather small and may reflect the fact that the study period did not cover eutrophic, but only mesotrophic and oligotrophic conditions. Isotopic signatures of fish species showed the same range and similar seasonal differences as those of the invertebrates indicating similar strengths of littoral-pelagic coupling for both organism groups.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by M.S. Figures were prepared by M.S. The first draft of the manuscript was written by M.S., and all authors commented and provided changes on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Raw data are published in the KonData repository, doi: 10.48606/110. as Sabel et al (2024) Stable isotope signatures of littoral invertebrates and fish in Lake Constance.

Declarations

Competing interest The authors declare that they have no competing interests as well as no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Ethical approval The authors declare that they have no competing or conflicting financial or non-financial interests. Fish sampling was carried out with a permit from the Regierungspräsidentium Tübingen (AktENZEICHEN 33–4/9220.51–3).

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